

Letter to the Editor

Profilin: A relevant aeroallergen?*To the Editor:*

Profilin is a panallergen that is present in all eukaryotic cells. It is one of the main causes of cross-sensitization between pollen and plant-derived foods.¹ As a food allergen, it usually induces oral allergy syndrome,¹ but its clinical relevance as a respiratory allergen remains unknown.

We sought to investigate the prevalence of nasal and bronchial responses to profilin in patients with pollen allergy with rhinitis, asthma, or both who were sensitized to profilin and also to measure the profilin content in different pollen extracts.

We report 28 patients with rhinitis symptoms compatible with seasonal allergic rhinitis, asthma, or both (23 patients sensitized to pollens and profilin and 5 control subjects sensitized to pollens with negative skin prick test responses and specific IgE results to profilin) enrolled from our outpatient clinic during the winter of 2009-2010. All patients received written information and signed a written informed consent form authorized by our institutional review board.

At the clinic, participants underwent skin prick testing with common aeroallergens and purified natural date palm profilin (Pho d 2; 50 µg/mL; ALK-Abelló, Madrid, Spain),² as well as fruit allergens (melon, peach, apple, plum, pear, kiwi, banana, and orange). Histamine and glycerol saline solution were used as controls (all from ALK-Abelló).

Nasal and bronchial tidal volume was measured in all patients after challenge testing by using a face mask with Pho d 2 extract (maximum Pho d 2, 100 µg/mL). Nasal response was assessed by means of acoustic rhinometry, for which the minimal transverse area (MTA) was measured. Results were considered to be positive when a decrease of 30% or greater in the MTA and bronchial response was detected by means of spirometry; positive results were all those reflecting a decrease of 20% or greater in FEV₁, as previously described.^{3,4} PC₂₀ methacholine values were also determined, as previously described.⁴

Specific serum IgE testing with the microarray technique (ISAC; Phadia, Uppsala, Sweden), which included rBet v 2, nOle e 2, rHev b 8, rMer a 1, and rPhl p 12, was performed in all patients.

Natural profilin (Pho d 2) was prepared by purifying a date palm extract by means of affinity chromatography with a Poly (L-proline)-Sephacrose. Purity (99%) was checked by means of SDS-PAGE, mass spectrometry, and amino acid analysis.²

An inhibition assay was developed with the ADVIA Centaur platform (Siemens, Berlin, Germany) to determine profilin content in different pollen extracts. This assay uses a reverse sandwich architecture with a monoclonal murine anti-human IgE antibody covalently bound to paramagnetic particles in the solid phase and capturing the sample IgE that reacts with biotin-labeled Pho d 2.⁵ In our inhibition assay a pool of patient sera sensitized to profilin was preincubated with known amounts of purified Pho d 2, as well as varying dilutions of the different pollen extracts analyzed. The 8 allergenic preparations were standardized from freeze-dried extracts provided by ALK-Abelló. They were analyzed for total protein content by using the Lowry method⁶ and for major allergen content by using specific ELISAs.^{7,8}

Table I displays participants' characteristics and test results. The mean age was 39 ± 14 years. Twenty-eight patients had positive specific IgE results to pollen showing a pattern of polysensitization to major pollen allergens.

Twenty-three patients had positive specific serum IgE results to all profilin proteins present in the microarray kit. All 5 control subjects had negative specific serum IgE results for profilin.

Twenty patients from the profilin-sensitized group were sensitized to at least 1 fruit allergen, and 11 presented with oral allergic syndrome to fruit.

In the profilin-sensitized group 16 (69%) patients had a positive methacholine challenge result (PC₂₀ <8 mg/mL) and a mean PC₂₀ value of 3.00 ± 2.45 mg/mL.

Seventeen (77%) patients had a positive specific bronchial challenge results with Pho d 2, and the mean PC₂₀ value was 4.52 ± 2.46 µg/mL. Eight of these 17 patients also had a positive nasal challenge result, and the mean MTA decrease was 41.8% ± 10.1%. Two (9%) patients had positive nasal challenge results only.

In the control group 4 of 5 patients had positive methacholine challenge results, with a mean PC₂₀ value of 3.62 ± 2.48 mg/mL. All 5 patients had negative nasal and bronchial challenge results (PC₂₀ >100 µg/mL) with Pho d 2.

Inhibition curves of the purified Pho d 2 were parallel in the range selected, thus allowing quantification of the amount of profilin equivalent in each of the extracts (data not shown). A summary of the determinations performed appears in Table II. The results show very high variability in the Pho d 2 content within the tested pollen extracts, with a range between 1 µg per vial in the *Betula alba* extract and 75 µg per vial in the *Lolium perenne* preparation. It can also be observed that the profilin amount present in these extracts is very low compared with the major allergen (Bet v 1, Lol p 5, Phl p 5, Ole e 1, and Pla l 1) content. The relative percentage of profilin with respect to the total amount of protein for each extract is shown in Table II.

In this study we provide further evidence supporting the concept that profilin can elicit nasal and bronchial responses in patients with pollen allergy who are also sensitized to profilin and therefore should be considered as a respiratory allergen. Nasal responses were lower than bronchial responses, likely because of low concentrations of allergen reaching the nasal mucosa. Nasal and bronchial challenge tests with natural and recombinant Bet v 1 were previously described by Godnic-Cvar et al.⁹ To our knowledge, ours is the first study to demonstrate that profilin can induce allergic respiratory symptoms. In the other part of the study, we demonstrated that the amount of profilin present in different pollen extracts is low compared with the quantity of the main allergens within each pollen studied. The profilin content in grasses is significantly higher than that present in trees or weeds. This higher prevalence in grasses supports and could explain the correlation between profilin sensitization and grass allergy.^{5,10} However, these findings do not indicate that a standardization strategy for grass vaccines based on the quantification of potential relevant minor allergens, such as profilin, could increase its efficacy in patients sensitized to grass pollen.

In conclusion, profilin can elicit respiratory symptoms in sensitized patients with rhinitis, asthma, or both. Therefore it

TABLE I. Summary of characteristics and test results

Patient no.	Age (y)/sex	Positive SPT response with profilin (mm)	Specific IgE to profilin	SPT with pollen	SPT with fruits	Methacholine PC ₂₀ (mg/mL)	FEV ₁ decrease (%) from baseline	Profilin PC ₂₀ (μg/mL)	Result	MTA decrease (%) from baseline	Result
1	49/M	9	Positive	G, P, C, O, Ph, Se, S, Pl	Me, Pe	9.60	36.2	<3.125	Positive	0	Negative
2	42/F	8	Positive	G, Cy, C, O, S, Pl	Me	1.12	1.1	>100	Negative	37	Positive
3	52/F	6	Positive	G, Cy, O, Pl	Me	>16	5.1	>100	Negative	51.9	Positive
4	45/F	10	Positive	G, C, P, O, Se, S, Pl, Ph	Me, B	0.94	40	8.05	Positive	32	Positive
5	37/M	9	Positive	G, Pl, O, Se, S, Pl	Pe, Me, B	0.98	21.8	6.06	Positive	41	Positive
6	22/M	6	Positive	G, Cy, Pl, O	Pe, Me	0.68	45.4	3.8	Positive	55.3	Positive
7	44/M	5	Positive	C, O, Ph, Se, S, Pl	Negative	7.20	3.1	>100	Negative	19.5	Negative
8	48/F	8	Positive	G, P, O, Se, Ph, S, Pl	Me	5.40	20.5	48.7	Positive	31	Positive
9	43/F	10	Positive	G, O, Se, P	Pe, B	12.43	0	>100	Negative	0	Negative
10	39/M	8	Positive	G, Cy, O, Se	Pe	>16	10.1	>100	Negative	0	Negative
11	37/M	6	Positive	G, C, P, Cy, O, Ph, Se, S	Negative	>16	36.06	70.3	Positive	59.2	Positive
12	19/M	5	Positive	G, P, C, O, Ph, Se, S, Pl, P	Me	>16	26.1	5.15	Positive	41.9	Positive
13	33/F	10	Positive	G, Cy, P, Cy, Ph, O, Se, S	Me	5.33	26.26	19.89	Positive	31.4	Positive
14	46/M	8	Positive	C, P, O, S	Me	1.90	19.3	11.2	Positive	0	Negative
15	20/F	9	Positive	G, Cy, P, C, O, Ph, Se, Pl	Pe, Me	1.91	30	2.41	Positive	39	Positive
16	41/M	9	Positive	G, Cy, C, O, Se, Ph, S	Pe	1.67	17.9	6.25	Positive	0	Negative
17	24/F	7	Positive	G, O, P, S, C, Ph, Pl, Pr	Me	0.52	34.17	8.73	Positive	0	Negative
18	46/M	7	Positive	G, Cy, P, C, O, Ph, Se, Pl	Me	2.58	19.50	6.25	Positive	0	Negative
19	18/F	7	Positive	G, Cy, C, O, Ph, Se	K	3.9	20.7	6.25	Positive	0	Negative
20	36/F	10	Positive	G, Cy, P, Ph, Se, S, O	Me	0.78	26.3	1.56	Positive	0	Negative
21	46/F	9	Positive	G, Cy, C, P, O, Ph, Se, S	Pe	5.40	27.3	3.125	Positive	0	Negative
22	44/M	7	Positive	G, Cy, C, O, Ph, Se, S, Pl	Negative	9.13	5.5	>100	Negative	0	Negative
23	29/F	9	Positive	G, Cy, P, C, O, Ph, S, Pr	B	7.70	21.07	<1.56	Positive	0	Negative
24	36/F	0	Negative	G, Cy, C, P, Ph, Se	Me, Pe	0.87	0	>100	Negative	0	Negative
25	35/M	0	Negative	G, Cy, P, C, O, Ph, S, Pr	Me, Pe, B	3.57	0	>100	Negative	0	Negative
26	25/M	0	Negative	G, C, O, Se, S, Ph	Negative	>16	0	>100	Negative	0	Negative
27	40/M	0	Negative	G, Cy, P, C, Se	Negative	6.89	0	>100	Negative	0	Negative
28	30/F	0	Negative	Cy, P, O, Se	Pe	3.15	0	>100	Negative	0	Negative

B, Banana; C, *Cynodon* species; Cy, cypress; F, Female; G, grass; K, kiwi; M, male; Me, melon; O, olive; P, *Platanus* species; Pe, peach; Ph, *Phragmites* species; Pl, *Plantago* species; Pr, *Parietaria* species; S, *Salsola* species; Se, *Secale* species; SPT, Skin prick test.

TABLE II. Determination of Pho d 2, total protein, and major allergen content in the 8 pollen extracts

Pollen extract	Pho d 2 (μg)	Total protein (mg)	Major allergen (μg)	Percentage of relative profilin content with respect to the total amount of protein in each extract
<i>Betula verrucosa</i>	1.1	2.27	410 Bet v 1	0.05
<i>Chenopodium album</i>	2.5	6.64	NM	0.04
<i>Lolium perenne</i>	75.0	8.17	1220 Lol p 5	0.80
<i>Olea europaea</i>	4.9	4.90	2473 Ole e 1	0.10
<i>Phleum pratense</i>	46.1	6.62	736 Phl p 5	0.70
<i>Plantago lanceolata</i>	1.8	7.67	33 Pla l 1	0.01
<i>Salsola kali</i>	4.8	13.0	NM	0.04

Data are presented as micrograms or milligrams per vial of freeze-dried extract. In *Chenopodium album* and *Salsola kali* extracts, the major allergen content was not measured. NM, Not measured.

should be considered a potentially relevant respiratory allergen when patients are exposed to high levels of grass pollen.

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